REMARKS

Applicants have carefully studied the Final Office Action mailed on May 5, 2004, which issued in connection with the above-identified application, and thank the Examiner and his Supervisor for the courtesy of considering a proposed amendment to the claims (discussed below). The present amendments and remarks are intended to be fully responsive to all points of rejection raised by the Examiner and are believed to place the claims in condition for allowance. Favorable reconsideration and allowance of the present claims are respectfully requested.

Pending Claims

Claims 2-5, 7-17, 19-29, 32, 39, and 42-45 are pending and at issue in the application.[1] Claims 2-5, 7-14, 42, and 43 have been temporarily withdrawn from consideration, but, as specified at page 2 of the previous Office Action dated August 12, 2003 (paper 20), could be rejoined with claims under examination upon determination of the allowable subject matter. Claims 29-32 and 39 have been rejected under 35 U.S.C. § 112, second paragraph, as being indefinite. Claims 15-17, 19-30, 32, 39, and 44 have been rejected under 35 U.S.C. § 103(a) as being obvious over the prior art.

Claims 29, 32 and 39 have been amended to correct formal defects. Applicants gratefully acknowledge the courtesy shown by the Examiner and the Examiner's Supervisor in reviewing the amendments to claims 29, 32 and 39 in an informal communication on September 1 and September 7, 2004. No new subject matter has been added as a result of these amendments, no new search is required, and no new issues are raised.

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[1] In the Office Action Summary, the Examiner states that claims 2-17, 19-29, and 45 are pending. Applicants respectfully note that claims 1, 6, 18, 30-31, 33-38, and 40-41 have been canceled in previous amendments, while claims 2-5, 7-17, 19-29, 32, 39, and 42-45 are pending.

35 U.S.C. §112, Second Paragraph, Rejections

In the Office Action, the Examiner has rejected claims 29-32 and 39 under 35 U.S.C. §112, second paragraph, as being indefinite for lack of active method steps that result in the production of infectious virus.

As claims 30-31 have been canceled, the rejection of these claims is rendered moot.

Claims 29, 32 and 39 have been amended to explicitly recite the results of practicing the method. The Examiner and the Examiner's Supervisor approved this amendment in an informal communication on September 1 and September 7, 2004.

In light of the foregoing, the rejection of the claims based upon 35 U.S.C. §112, second paragraph, is believed to be overcome and withdrawal of such is kindly requested.

35 U.S.C. §103(a) Rejections

In the Office Action, the Examiner has maintained the rejection of claims 15-17, 19-30, 32, 39, and 44 under 35 U.S.C. §103(a) as being obvious over Hoffmann dissertation (1997) and Neumann *et al.* (Proc. Natl. Acad. Sci., 1999, 96: 9345-50) and has rejected claim 45 as being obvious over Hoffmann and Neumann *et al.* and further in view of Pleshka *et al.* (J. Virol., 1996, 70: 4188-92). The Examiner contends that, knowing that Neumann *et al.* generated infectious influenza virus by a plasmid-based system and the benefit of adding more protein expressing plasmids to the transfection and knowing that multi-plasmid transfections are complex, one of ordinary skill in the art at the time of the invention would be motivated to use the plasmid of Hoffmann to reduce the number of plasmids required to transfect and save time in cloning with the expectation of success, because the promoter elements used by Hoffmann are the same as used by Neumann *et al.*

As claim 30 has been canceled, the rejection of this claim is rendered moot. With respect to the remaining claims, the rejection is respectfully traversed for the reasons provided below.

The present claims call for a set of dual promoter pol I-pol II plasmids (each comprising one influenza viral genomic segment). This set of plasmids, when co-introduced into a host cell in the absence of any helper virus or additional viral protein-expressing plasmids, produce an infectious influenza virus.[2]

As specified in the attached Expert Opinion Declarations of Dr. Robert G. Webster (Exhibit A, section 15) and Dr. Richard J. Webby (Exhibit B, section 9), the Hoffmann dissertation describes single pol I-pol II plasmids carrying heterologous reporter genes such as CAT and GFP. The Hoffmann dissertation does not disclose or suggest making even a single pol I-pol II plasmid encoding a viral gene segment, or that such a single plasmid would work in the context of influenza virus rescue, much less a full set of such plasmids capable of reconstituting an infectious influenza virus in the absence of a helper virus. The Hoffmann dissertation discloses transcription of a single reporter mRNA and expression of a reporter protein using host cell transcription/translation machinery and replication of a single reporter RNA in the presence of viral polymerase proteins supplied by a helper virus (FPV, pp. 114-115). Such disclosure does not provide any teaching or suggestion or a reasonable expectation of success that eight influenza viral RNAs can be transcribed/replicated using dual promoter pol I-pol II plasmids to achieve generation of an infectious viral particle. Indeed, as Dr. Webby states in section 9 of his Expert Opinion Declaration:

> The pol I-pol II reporter gene construct described in the Hoffmann thesis is an interesting curiosity. However, even if one were to extrapolate from this construct to a construct with a viral gene instead of a reporter gene, there remain vast uncertainties as to whether the expression of vRNA and mRNA from that construct would yield functional RNA molecules. To then leap to the 8-plasmid dual pol I-pol II promoter system of the present invention goes orders of magnitude beyond what the Hoffmann dissertation actually teaches or suggests. Each of the 8 dual promoter plasmid constructs introduces a degree of uncertainty, further compounded when one omits a helper virus.

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[2] Claims are pending, albeit withdrawn, directed to each individual construct.

The Neumann *et al.* article does not teach the pol I-pol II dual promoter plasmids, but teaches separate pol I-only and pol II-only plasmids. Nothing in Neumann suggests the desirability of substituting the pol I-only and pol II-only plasmids for Hoffmann's pol I-pol II dual promoter plasmids, nor does anything in Hoffmann provide a basis for using the pol I-pol II plasmids in the Neumann plasmid-based system for generation an infectious influenza virus.

As specified in detail in the attached Expert Opinion Declarations of Dr. Webster (section 13) and Dr. Webby (sections 11 and 13), the separation of pol I and pol II promoters on different plasmids lies at the very core of the Neumann *et al.* plasmid system as this system attempts to recapitulate the life cycle of an influenza virus by allowing regulation of viral protein expression separately from viral genome segment replication. In fact, in its most efficient (high yield) versions, the system of Neumann *et al.* uses, in addition to protein expression (pol II) plasmids encoding PB1, PB2, PA, and NP influenza proteins, extra protein expression plasmids encoding HA, NA, M1, M2, and NS2 proteins (making the total desirable number of transfected plasmids 17 instead of 12; see, p. 9347 [¶ bridging left and right col.] and Table 1 at p. 9348 of Neumann *et al.*). Based on this disclosure, a person skilled in the art would be persuaded to increase, not to decrease, the number of plasmids in a plasmid-based influenza expression system. The optimal use of the reverse genetic system disclosed in Neumann *et al.* also relies on the ability to provide specified amounts of each viral protein by transfecting different amounts of each of the protein expression (pol II) plasmids (see, *e.g.*, p. 9347 left col., ¶¶ 3-4 of Neumann *et al.*).

In contrast to Neumann *et al.*, the reverse genetic system of the present invention contains fewer plasmids (*e.g.*, eight for influenza A virus) which can be transfected in roughly equal amounts (see, *e.g.*, p. 72, ll. 11-12 of the present specification). Most importantly, the dual pol I-pol II promoter reverse genetic system of the present invention is based on a different principle of coordination of viral replication and protein expression as compared to the single promoter plasmid system of Neumann *et al.* In the dual pol I-pol II promoter system of the present invention, the vRNA/cRNA and mRNA syntheses occur from the same plasmid and cannot be

separately controlled. This creates additional spatial and temporal hurdles for regulation of transcription and replication, which are not present either in the Neumann *et al.* system or in nature. As specified in section 14 of the Expert Opinion Declaration of Dr. Webby, such spatial and temporal hurdles stem, for example, from the fact that pol I-mediated transcription (vRNA/cRNA synthesis) takes place in the nucleolus, while pol II-mediated transcription (mRNA synthesis) takes place in a different site in the nucleus. Since, at the time of the invention, it was not known whether mRNA and vRNA/cRNA syntheses occur on pol I-pol II plasmids at the same time, the ability of these plasmids to produce both mRNA and vRNA/cRNA appeared uncertain. Also, it appeared uncertain that pol I- and pol II-directed transcription (involving assembly of huge molecular complexes and conformational changes of the template) can occur successfully on the same artificial construct in the absence of natural viral regulatory mechanisms. Indeed, mRNA transcripts derived from pol I-pol II plasmids contain pol I promoter and terminator sequences in their 5' and 3' non-coding regions. These sequences (not present in the Neumann *et al.* system) are sites where pol I transcription and termination factors bind. One could therefore envision that binding of these factors to pol I promoter and terminator regions in the pol I-pol II plasmids may result in reduction in pol II-mediated transcription. The interaction of pol I promoter and terminator sequences with influenza sequences in mRNA derived from pol I-pol II plasmids was also unpredictable at the time of the invention and one could envision that it could possibly affect mRNA stability and translation efficiency leading to a lower abundance of some or all mRNAs. Neither Neumann *et al.* nor the Hoffmann dissertation provide any suggestion that such spatial and temporal hurdles for regulation of transcription and replication can be overcome with respect to each viral segment and result in the production of an infectious virus.

Indeed, as specified in the attached Expert Opinion Declarations of Dr. Webster (sections 14 and 16) and Dr. Webby (section 14), having a complete knowledge of the Neumann *et al.* reference and the Hoffmann dissertation, leading scientists in the field were highly skeptical that

the dual pol I-pol II promoter plasmid system of the present invention can produce an infectious virus. Thus, Dr. Webster, a leading world expert in the field of influenza virology, states in section 14 of his Declaration that "when Dr. Hoffmann came up with the idea of the dual pol I-pol II promoter system, everyone in the laboratory, including myself, was very skeptical that such a system could produce an infectious virus". Similarly, Dr. Webby concludes section 14 of his Declaration by stating that "the success of the present invention came as a great surprise".

Taken together, neither the Hoffmann dissertation nor Neumann *et al.* article provide any expectation of success, much less any motivation to be combined with the other reference or to modify the disclosed compositions. These references in combination do not in any way suggest a dual promoter pol I-pol II plasmid encoding an influenza viral gene segment, much less the claimed composition of a set of dual promoter pol I-pol II plasmids for the generation of infectious influenza viruses from cloned viral cDNA as recited in the present claims. As Dr. Webby states in section 17 of his Declaration, "there was no way to know whether the pol I-pol II plasmid approach of the present invention would work, until Dr. Hoffmann showed that it worked". As further specified in section 19 of the Declaration of Dr. Webster

> [T]he present invention represents a significant breakthrough both in the field of basic virology of negative stranded RNA viruses and in the field of influenza vaccine production. I strongly disagree with the Examiner's conclusion that the present invention is obvious over the Hoffmann dissertation and Neumann *et al.* article. Perhaps viewed from the vantage of Dr. Hoffmann's success it seems obvious to introduce the viral gene segments into pol I-pol II dual promoter plasmids. However, none of us - including myself, one of the leading experts in influenza in the world, thought so at the time... It took extraordinary vision to employ the dual pol I-pol II promoter system for all of the viral segments. In my view, such vision, and the results obtained, are the antithesis of obviousness.

The only conclusion one can come to in view of the opinion of Drs. Webster and Webby, two leading world experts in the field of influenza virology and recombinant vaccines whose Expert Opinion Declarations are attached to this Response, is that the present invention is not obvious over the cited prior art. Affidavits of those skilled in the art have been held to constitute

factual evidence of the level of skill in the art. *E.g., In re Piasecki*, 223 U.S.P.Q. at 789; *In re Oelrich*, 198 U.S.P.Q. 210, 214-15. Such affidavits constitute competent evidence that cannot be ignored. *See e.g., Ashland Oil, Inc. v. Delta Resins & Refractories, Inc.*, 227 U.S.P.Q. 657, 674-75 (Fed. Cir. 1985). Only an improper hindsight reconstruction could rebut the experts' position. It is well settled however, that such hindsight reconstruction is an error.[3]

The absence of a motivation to combine the Neumann *et al.* and Hoffmann references and the absence of obviousness to a person of ordinary skill in the art at the time the present application was filed also follows from the following additional disclosures.

Fodor *et al.*, J. Virol., 1999, 73: 9679-9682 (cited in the Information Disclosure Statement). This article was published almost simultaneously with Neumann *et al.* and reported a similar approach in which influenza virus was generated by cloning cDNAs encoding vRNAs under the control of the human RNA pol I promoter. The hepatitis delta virus ribozyme was used to generate the 3' ends of the viral transcripts. Transfection of the pol I/ribozyme plasmids into mammalian cells, together with protein expression plasmids for the polymerase and NP proteins, yielded infectious virus.

Similarly to Neumann *et al.*, Fodor *et al.* used, in addition to vRNA-encoding plasmids, viral protein expression plasmids. In contrast to Neumann *et al.*, when discussing at the end of the article how to improve the efficiency of viral rescue in their recombinant system, Fodor *et al.* acknowledge that reduction of the number of plasmids may be useful. However, in contrast to the present invention, they suggest to reduce the number of plasmids not by creating pol I-pol II dual promoter constructs but by creating cell lines stably expressing essential proteins for encapsidation, transcription, and replication of viral genomic RNA (PB1, PB2, PA, and NP) (see p. 9681, left col., ¶ 2).

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[3] *In re Dow Chemical Co.*, 5 USPQ2d 1529, 1532 (Fed. Cir. 1988); *In re Fritch*, 23 USPQ2d 1780, 1784 (Fed. Cir. 1992); *W.L. Gore & Assocs., Inc. v. Garlock, Inc.*, 721 F.2d 1540, 1552 (Fed. Cir. 1983); *Ex parte Obukowicz*, 27 USPQ2d 1063 (BPAI 1993).

The authors of Fodor *et al.* published their articles in 1999 and were among the leaders in the field of recombinant generation of influenza viruses. While they may have recognized the importance of reducing the number of plasmids to increase the efficiency of viral rescue, they were still not able to appreciate the usefulness of the dual promoter pol I-pol II plasmid disclosed in the Hoffmann dissertation (1997) to solve the problem of reducing the number of plasmids. Clearly, a person of average skill in the art would be even less able or motivated to do so.

Neumann and Kawaoka, Rev. Med. Virol., 2002, 12: 13-30 (attached as Exhibit C). This is a recent review by the two senior authors of the Neumann *et al.* article in which they state as follows (p. 22, ¶ bridging left and right col.; emphasis added):

> The RNA polymerase I system for the generation of influenza A virus requires the cotransfection of 12-17 plasmids and may limit the efficient generation of virus to cell lines that can be transfected with high efficiencies. To reduce the number of plasmids, Hoffmann et al. explored a *different* route, cloning cDNA encoding vRNA in positive orientation between an RNA polymerase II promoter derived from cytomegalovirus and a polyadenylation signal. They inserted this cassette in negative orientation between RNA polymerase I promoter and terminator sequence. Thus, transfection of the plasmid into eukaryotic cells yields negative-sense vRNA (synthesised by RNA polymerase I) and positive-sense mRNA for protein expression (synthesised by RNA polymerase II) from one template. Consequently, cotransfection of separate protein expression plasmids is no longer necessary, thereby reducing the number of plasmids required for virus generation. The efficiency of virus generation in this system is comparable to that of the RNA polymerase I system, although the fact that both protein expression and vRNA synthesis are achieved from the same template reduces the flexibility of the system. For example, the RNA polymerase I/II system is not suitable for the formation of virus-like particles lacking or containing lethal mutations in one or more viral segments in studies of viral protein functions or gene delivery (see below). Nonetheless, both the RNA polymerase I and the RNA polymerase I/II systems provide excellent tools for numerous applications.

As follows from this citation, Neumann and Kawaoka consider the present invention a noteworthy "different route" as compared to the recombinant system disclosed in Neumann *et al.* article. Furthermore, they question the usefulness of the present invention with respect to some

applications, which clearly reflects that they do not consider it an obvious variation of Neumann *et al.*

The fact that the present invention constitutes a significant advance which by no means is obvious in light of Neumann *et al.* is further emphasized in a recent article by de Wit *et al.* (Virus Res., 2004, 103: 155-161; attached as Exhibit D) (emphasis added):

(p. 155, right col.)

> Neumann *et al.* reported that upon co-expression of five additional plasmids encoding the hemagglutinin (HA), neuraminidase (NA), matrix proteins 1 and 2 (M1 and M2) and non-structural protein 2 (NS2), virus titers in the supernatants could be increased up to $5x10^7$ pfu/ml. An elegant modification of these 12 and 17-plasmid systems came from Hoffmann *et al.* who implemented bidirectional vectors to reduce the number of transfected plasmids to eight. With this system, the negative-stranded vRNA and the positive-stranded mRNA can be synthesized from the same plasmid and virus titers up to $2x10^7$ were reported (Hoffmann *et al.*, 2000). The ability to produce recombinant influenza A virus rapidly and in such high titers will greatly facilitate future influenza virus research.

(p. 160, left col., beginning of Discussion)

> A bidirectional 8-plasmid transcription system that was first described for influenza virus A/WSN/33 (Hoffmann *et al.*, 2000) was found to be superior to our in-house unidirectional 12-plasmid transcription system. A large difference was observed in the amount of virus produced from 293T cells transfected with constructs encoding influenza virus A/PR/8/34 and A/WSN/33...

As further summarized in sections 16-17 of the Expert Opinion Declaration of Dr. Webster and section 15 of the Expert Opinion Declaration of Dr. Webby, the dual pol I-pol II promoter plasmid system of the present invention turned out to be not only much simpler and more reproducible than the system of Neumann *et al.*, because it significantly decreased the number of plasmids for transfection and eliminated the need to adjust the relative amount of each plasmid, but efficient as well. Efficiency of the plasmid system of the present invention is

another unexpected benefit, especially in light of Neumann *et al.* demonstrating greater efficiency with more plasmids.

Also, in contrast to the Neumann *et al.* system, which for a long time was capable of efficiently producing infectious virus only in 293T cells, the dual pol I-pol II promoter system of the present invention efficiently generated infectious viral particles in monkey Vero cells. This is not a trivial difference. 293T cells are transformed cells that are not approved by WHO for vaccine seed production. Vero cells are approved for vaccine seed production. Thus, at the time of the present invention, the Neumann *et al.* system would have been regarded as not yet practical. Nothing in Hoffmann's thesis indicates this particular characteristic of the dual pol I-pol II promoter system.

Non-obviousness of the of the dual pol I-pol II promoter plasmid system of the present invention is further demonstrated by its unmatched usefulness for the fast and easy generation of influenza vaccines which addresses a raging need for rapid influenza vaccine response, particularly with the expectation of a worldwide pandemic in the near term. As specified in sections 17-18 of the Declaration of Dr. Webster and section 16 of the Declaration of Dr. Webby, more and more investigators around the world are using the dual pol I-pol II promoter reverse genetic system of the present invention to generate recombinant influenza vaccines. For example, the use of the 8-plasmid dual pol I-pol II promoter system of the present invention allowed researchers at St. Jude Children's Research Hospital to produce a vaccine to the deadly avian H5N1 influenza virus A/Hong Kong/213/03, which caused human infections and lead to a WHO pandemic alert on February 19, 2003. The vaccine was produced in Good Manufacturing Practice (GMP)-grade facilities *in less than 4 weeks* from the time of virus isolation (see Webby *et al.*, Lancet, 2004, 363: 1099-103; attached as Exhibit E). The ability to produce a candidate reference virus in such a short period of time has set a new standard for rapid response to emerging infectious disease threats and has clearly demonstrated the superior usefulness of the dual pol I-pol II promoter system of the present invention for influenza vaccine development.

The same technologies and procedures are currently being used to create reference vaccine viruses against the 2004 H5N1 viruses circulating in Asia.

In sum, neither the Hoffmann dissertation nor Neumann *et al.* article provide any expectation of success or suggestion to be combined with the other reference. Even if forcibly combined, Neumann *et al.* and the Hoffmann dissertation do not disclose or suggest modifying the disclosed compositions to generate a set of dual promoter pol I-pol II plasmids for the generation of infectious influenza viruses from cloned viral cDNA as recited in the present claims.

In the Office Action, claim 45 stands rejected under 35 U.S.C. §103(a) as being obvious over Hoffmann and Neumann *et al.* and further in view of Pleshka *et al.* (J. Virol., 1996, 70:4188-92) who teach that ribozymes can be used to generate specific ends of influenza segments. As specified above, even if combined, Neumann *et al.* and the Hoffmann dissertation do not disclose or suggest the dual promoter pol I-pol II plasmid composition which generates infectious influenza viruses from cloned viral cDNA as recited in claim 45. The knowledge of the fact that ribozymes can be used to generate specific ends of influenza segments does not provide any missing link which would allow one skilled in the art to arrive at the present invention.

In light of the foregoing arguments, it is respectfully submitted that pending claims are not obvious over the cited art. Reconsideration and withdrawal of the obviousness rejection is believed to be in order.

CONCLUSION

Applicants request entry of the foregoing amendments and remarks in the file history of this application. In view of the above amendments and remarks, it is respectfully submitted that claims 2-5, 7-17, 19-29, 32, 39, and 42-45 are now in condition for allowance and such action is

earnestly solicited. If the Examiner believes that a telephone conversation would help advance the prosecution in this case, the Examiner is respectfully requested to call the undersigned agent at (212) 527-7634. The Examiner is hereby authorized to charge any additional fees associated with this response to our Deposit Account No. 04-0100.

Respectfully submitted,

Dated: November 5, 2004

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